Amendments to the Specification

Please add the following new paragraphs at page 12, after the first full paragraph (i.e., after paragraph [0049] of the published application):

In another embodiment, the composition comprises 2,6-diisopropylphenol, polyethylene glycol 400, poloxamer 188 and propylene glycol. This composition can comprise: (a) propofol as described above; (b) about 1 to about 25%, about 1 to 15%, about 2 to 10%, about 2 to 8%, or about 2 to about 6% (w/v) polyethylene glycol 400, for example, about 3 to about 6% or about 4 to about 6% (w/v) polyethylene glycol 400; (c) about 0.5 to about 25%, about 0.5 to 15%, about 0.5 to 10%, about 0.5 to 8%, or about 0.5 to about 5% (w/v) propylene glycol, for example, about 0.5 to about 3% or about 0.5 to about 2% (w/v) propylene glycol; (d) about 1 to about 25%, about 1 to 15%, about 4 to 12%, about 5 to 10%, or about 6 to about 8% (w/v) poloxamer 188, for example, about 5 to about 9% or about 6 to about 7% (w/v) poloxamer 188; and (e) water. Optionally, benzyl alcohol may be added to this composition in concentrations up to 5%, up to 4%, up to 3%, up to 2%, up to 1% or up to 0.5%.

In other embodiments, the composition consists essentially of:

(1) water, 2,6-diisopropylphenol, poloxamer 188, polyethylene glycol 400, propylene glycol, optionally, a tonicity modifier, and, optionally, a pH modifier, or stabilizer (e.g., antioxidant such as cysteine, chelating agent such as EDTA, or other such as citric acid);

(2) water, 2,6-diisopropylphenol, poloxamer 188, polyethylene glycol 400, propylene glycol, and, optionally, citric acid or a salt thereof; or

(3) water, 2,6-diisopropylphenol, poloxamer 188, polyethylene glycol 400, propylene glycol, optionally, citric acid or a salt thereof, and, optionally, a tonicity modifier.

(4) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 8% (w/v), polyethylene glycol 400 at about 4% (w/v), and propylene glycol at about 1% (w/v).

(5) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 6% (w/v), polyethylene glycol 400 at about 6% (w/v), and propylene glycol at about 1% (w/v).

(6) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 6% (w/v), polyethylene glycol 400 at about 4% (w/v), and propylene glycol at about 2% (w/v).

(7) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 8% (w/v), polyethylene glycol 400 at about 3% (w/v), and propylene glycol at about 1% (w/v).

(8) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 7% (w/v), polyethylene glycol 400 at about 4% (w/v), and propylene glycol at about 1% (w/v).

(9) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 7% (w/v), polyethylene glycol 400 at about 3% (w/v), and propylene glycol at about 1% (w/v).

(10) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 6% (w/v), polyethylene glycol 400 at about 4% (w/v), and propylene glycol at about 1% (w/v).

(11) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 8% (w/v), polyethylene glycol 400 at about 2% (w/v), and propylene glycol at about 1% (w/v).

In another embodiment, the composition comprises 2,6-diisopropylphenol, polyethylene glycol 400, poloxamer 188, propylene glycol, and citric acid. This composition can comprise: (a) propofol as described above; (b) about 1 to about 25%, about 1 to 15%, about 2 to 10%, about 2 to 8%, or about 2 to about 6% (w/v) polyethylene glycol 400, for example, about 3 to about 6% or about 4 to about 6% (w/v) polyethylene glycol 400; (c) about 0.5 to about 25%, about 0.5 to 15%, about 0.5 to 10%, about 0.5 to 8%, or about 0.5 to about 5% (w/v) propylene glycol, for example, about 0.5 to about 3% or about 0.5 to about 2% (w/v) propylene glycol; (d) about 1 to about 25%, about 1 to 15%, about 4 to 12%, about 5 to 10%, or about 6 to about 8% (w/v) poloxamer 188, for example, about 5 to about 9% or about 6 to about 7% (w/v) poloxamer 188; (e) about 0.5 to 1% citric acid, about 0.5 to 4% citric acid, about 1 to 3% citric acid, about 2 to 5% citric acid, about 1 to 2% citric acid, and (f) water. Optionally, benzyl alcohol may be added to this composition in concentrations up to 5%, up to 4%, up to 3%, up to 2%, up to 1% or up to 0.5%.

In another embodiment, the composition comprises 2,6-diisopropylphenol, polyethylene glycol 400, and poloxamer 188. This composition can comprise: (a) propofol as described above; (b) about 1 to about 25%, about 1 to 15%, about 2 to 10%,

about 2 to 8%, or about 2 to about 6% (w/v) polyethylene glycol 400, for example, about 3 to about 6% or about 4 to about 6% (w/v) polyethylene glycol 400; (c) about 1 to about 25%, about 1 to 15%, about 4 to 12%, about 5 to 10%, or about 6 to about 8% (w/v) poloxamer 188, for example, about 5 to about 9% or about 6 to about 7% (w/v) poloxamer 188; and (e) water. Optionally, benzyl alcohol may be added to this composition in concentrations up to 5%, up to 4%, up to 3%, up to 2%, up to 1% or up to 0.5%.

In other embodiments, the composition consists essentially of:

(1) water, 2,6-diisopropylphenol, poloxamer 188, polyethylene glycol 400, optionally, a tonicity modifier, and, optionally, a pH modifier, or stabilizer (e.g., antioxidant such as cysteine, chelating agent such as EDTA, or other such as citric acid;

(2) water, 2,6-diisopropylphenol, poloxamer 188, polyethylene glycol 400, and, optionally, citric acid or a salt thereof; or

(3) water, 2,6-diisopropylphenol, poloxamer 188, polyethylene glycol 400, optionally, citric acid or a salt thereof, and, optionally, a tonicity modifier.

(4) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 8% (w/v), and polyethylene glycol 400 at about 4% (w/v).

(5) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 8% (w/v), and polyethylene glycol 400 at about 3% (w/v).

(6) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 7% (w/v), and polyethylene glycol 400 at about 4% (w/v).

(7) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 7% (w/v), and polyethylene glycol 400 at about 3% (w/v).

(8) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 6% (w/v), and polyethylene glycol 400 at about 6% (w/v).

(9) water, 2,6-diisopropylphenol at about 1% (w/v), poloxamer 188 at about 9% (w/v), and polyethylene glycol 400 at about 2% (w/v).

Another composition comprises 2,6-diisopropylphenol, polyethylene glycol 400, and a purified poloxamer with an average molecular weight range between about 7600 and 9500. This composition can comprise: (a) propofol as described above; (b) about 1 to about 25%, about 1 to 15%, about 2 to 10%, about 2 to 8%, or about 2 to about 6% (w/v) polyethylene glycol 400, for example, about 3 to about 6% or about 4 to about 6% (w/v) polyethylene glycol 400; (c) about 1 to about 25%, about 1 to 15%, about 4 to 12%, about 5 to 10%, or about 6 to about 8% (w/v) purified poloxamer with an average molecular weight range between about 7600 and 9500, for example, about 5 to about 9% or about 6 to about 7% (w/v) purified poloxamer with an average molecular weight range between about 7600 and 9500; and (e) water.

Another composition comprises 2,6-diisopropylphenol, polyethylene glycol 400, and a purified poloxamer with an average molecular weight range between about 7600 and 9000. Another composition comprises 2,6-diisopropylphenol, polyethylene glycol 400, and a purified poloxamer with an average molecular weight range between about 8000 and 9000. Another composition comprises 2,6-diisopropylphenol, polyethylene glycol 400, and a purified poloxamer with an average molecular weight range between about 8000 and 8500.

In another embodiment, the composition comprises 2,6-diisopropylphenol, polyethylene glycol 400, a purified poloxamer with an average molecular weight range

between about 7600 and 9500 and propylene glycol. This composition can comprise: (a) propofol as described above; (b) about 1 to about 25%, about 1 to 15%, about 2 to 10%, about 2 to 8%, or about 2 to about 6% (w/v) polyethylene glycol 400, for example, about 3 to about 6% or about 4 to about 6% (w/v) polyethylene glycol 400; (c) about 0.5 to about 25%, about 0.5 to 15%, about 0.5 to 10%, about 0.5 to 8%, or about 0.5 to about 5% (w/v) propylene glycol, for example, about 0.5 to about 3% or about 0.5 to about 2% (w/v) propylene glycol; (d) about 1 to about 25%, about 1 to 15%, about 4 to 12%, about 5 to 10%, or about 6 to about 8% (w/v) purified poloxamer, for example, about 5 to about 9% or about 6 to about 7% (w/v) purified poloxamer; and (e) water.

In another embodiment, the composition comprises 2,6-diisopropylphenol, polyethylene glycol 400, a purified poloxamer with an average molecular weight range between about 7600 and 9000 and propylene glycol. In another embodiment, the composition comprises 2,6-diisopropylphenol, polyethylene glycol 400, a purified poloxamer with an average molecular weight range between about 8000 and 9000 and propylene glycol. In another embodiment, the composition comprises 2,6-diisopropylphenol, polyethylene glycol 400, a purified poloxamer with an average molecular weight range between about 8000 and 8500 and propylene glycol.

In yet another embodiment, the composition comprises polyethylene glycol (e.g., PEG-400) and poloxamer (e.g., Poloxamer 237). This composition can comprise (a) propofol as described above; (b) about 2 to about 30%, about 3 to about 20%, about 3 to 15%, about 3 to 12%, or about 3 to about 9% (w/v) PEG-400, for example, about 3 to about 7% or about 5 to about 7% (w/v) PEG-400; (c) about 1 to about 25%, about 1 to 15%, about 1 to 10%, about 1 to about 5%, or about 1 to about 3% (w/v) Poloxamer 237,

for example, about 1 to about 2% or about 1.1 to about 1.5% (w/v) Poloxamer 237; and (d) water.

In alternative embodiments, the composition also consist essentially of:

(1) water, 2,6-diisopropylphenol, polyethylene glycol 400, Poloxamer 237, optionally, a tonicity modifier, and optionally, a pH modifier;

(2) water, 2,6-diisopropylphenol, polyethylene glycol 400, Poloxamer 237, optionally, and, optionally, citric acid or a salt thereof; or

(3) water, 2,6-diisopropylphenol, polyethylene glycol 400, Poloxamer 237, optionally, citric acid or a salt thereof, and, optionally, a tonicity modifier.

Another composition of the invention comprises polysorbate (e.g., polyoxyethylene 20 sorbitan monooleate), propylene glycol, polyethylene glycol (e.g., PEG-400), and poloxamer (e.g., Poloxamer 188). This composition can comprise (a) propofol as described above; (b) about 0.5 to about 25%, about 0.5 to 15%, about 1 to 10%, or about 1 to about 5% (w/v) polyoxyethylene 20 sorbitan monooleate, for example, about 1 to about 3% or about 1 to about 2% (w/v) polyoxyethylene 20 sorbitan monooleate; (c) about 0.5 to about 25%, about 0.5 to 15%, about 0.5 to 10%, about 0.5 to about 5%, about 0.5 to about 3%, about 0.5 to about 2%, about 0.5 to about 1%, or about 1 to about 3% (w/v) propylene glycol, for example, about 1 to about 2% (w/v) propylene glycol; (d) about 1 to about 30%, about 1 to about 20%, about 2 to 15%, or about 2 to about 8% (w/v) PEG-400, for example, about 3 to about 6% or about 4 to about 5% (w/v) PEG-400; (e) about 1 to about 25%, about 1 to 15%, about 2 to 10%, or about 2 to about 8% (w/v) Poloxamer 188, for example, about 3 to about 7% or about 4.5 to about 5.5% (w/v) Poloxamer 188; and (f) water. In some embodiments, this composition

further comprises citric acid or a salt thereof. Citric acid can be present in the compositions in concentrations of at least about 0.05 percent (w/v) such as about 0.05 to about 5%, about 0.1 to about 3%, about 0.1 to about 1% (w/v), for example, about 0.1 to about 0.5% or about 0.1 to about 0.2%, or 0.15%(w/v).

These compositions may alternatively consist essentially of:

(1) water, 2,6-diisopropylphenol, polyoxyethylene 20 sorbitan monooleate, propylene glycol, polyethylene glycol 400, Poloxamer 188, optionally, a tonicity modifier, and optionally, a pH modifier;

(2) water, 2,6-diisopropylphenol, polyoxyethylene 20 sorbitan monooleate, propylene glycol, polyethylene glycol 400, Poloxamer 188, and, optionally, citric acid or a salt thereof; or

(3) water, 2,6-diisopropylphenol, polyoxyethylene 20 sorbitan monooleate, propylene glycol, polyethylene glycol 400, Poloxamer 188, optionally, citric acid or a salt thereof, and, optionally, a tonicity modifier.

In some embodiments, the composition contains benzyl alcohol. In some compositions benzyl alcohol may provide added antimicrobial activity. Benzyl alcohol concentrations can be below 5% w/v, below 4% w/v, below 3% w/v, below 2% w/v, below 1% w/v, below 0.5% w/v, or at 0.45% w/v.

Please add the following new paragraphs at page 38, above the title "8. REFERENCES CITED":

7.5 Further Aqueous Propofol Examples

Example 1

A propofol containing composition (Formulation C) was prepared as follows. Approximately 500 mg PEG-400, 350 mg PEG-40 stearate, and 35 mg polyoxyethylene 20 sorbitan monooleate were added to a glass vessel. Purified water was added followed by 100 mg propofol. Water was added as necessary to bring the total volume to 10 milliliters. The mixture was stirred at room temperature using a magnetic stirring bar for at least 4 hours over a 24-hour period. The resulting composition was substantially transparent to the naked eye but slightly hazy.

Laser Light Scattering (LLS) particle size analysis was performed using a Zetasizer 3000HS (Malvern Instruments Inc., Southborough, Mass.). Particle size was determined to be less than approximately 100 nanometers.

Example 2

A propofol containing composition (Formulation D) was prepared as follows. Approximately 300 mg Poloxamer 237 and 600 mg PEG-400 were added to a glass vessel. Purified water was added followed by 100 mg propofol. Water was added as necessary to bring the total volume to 10 milliliters. The mixture was stirred at room temperature using a magnetic stirring bar for at least 4 hours over a 24-hour period. The resulting composition was clear to the naked eye with no visible solids present.

Example 3

A propofol containing composition (Formulation F) was prepared as follows. 3.0 g polyoxyethylene 20 sorbitan monooleate, 2.9 g propylene glycol, 8.0 g PEG-400, 10.0 g Poloxamer 188, and 0.4 g citric acid were added to a 250 mL volumetric flask.

Deionized water was added to the 150 mL marker and the contents of the flask were stirred for 3 hours. Additional deionized water was added to bring the total volume to 197.8 mL and the solution was stirred for one hour. 2.2 mL of 100% pure propofol was added to the flask and the contents of the flask were stirred for at least 8 hours (i.e., until all of the propofol droplets had dissolved). The mixture was filtered through a PVDF filter with a 0.2 micron pore size. The resulting composition was clear to the naked eye. HPLC analysis indicated that less than 1% of propofol was retained by filtration. Since the HPLC assay had a 1-2% variation, this less than 1% loss is not considered significant. Laser Light Scattering (LLS) particle size analysis was performed using a Zetasizer 3000HS (Malvern Instruments Inc., Southborough, Mass.) Particle size was determined to be approximately 20 to 100 nanometers. Physical stability of Formulation F was monitored by measuring mean particle size over the course of a 4 week study. Mean particle size was initially measured as 89±6 nanometers. A sample of Formulation F was held at 60°C for 4 weeks. At the end of the time period, mean particle size of Formulation F was 84±6 nanometers.

Example 4

Propofol containing Formulations C, D and F were prepared as in Examples 1, 2 and 3, respectively. The compositions were separately sealed in glass vials. The compositions then were subjected to a variety of environmental conditions. Reverse phase HPLC was used as an indicator of propofol and excipient chemical stability. HPLC conditions are shown in Table 4 below.

TABLE 4. HPLC Conditions

Column	Chromolith Performance RP-18e (Merck Kga) 4.6 x 100 mm
Mobile Phase	45% 50 mM KPO_4; pH 2.5; acetonitrile
Flow Rate	4.5 mL/min
Temperature Column Sample	 35 °C Ambient
Injection Volume	15 microliters
Run Time	5 minutes
Detection	UV, 272 nm

Prior to HPLC analysis, compositions were held at the indicated conditions for 4 weeks. HPLC was also performed on initially formed compositions. The percent of propofol degradation increase after 4 weeks is summarized in Table 5 below.

TABLE 5. Increase in the Percent of Total Propofol Degradates after 4 weeks.

Formulation	25°C	40°C
C	0.50	3.7
D	0.1	0.64
F	None detected	0.07

Analysis of degradates is the most sensitive way to gauge stability of relatively stable materials, such as the present propofol compositions, over a short period of time. The temperature increase from 25 to 40°C, the latter temperature representing accelerated conditions, was responsible for increasing amounts of oxidation in each case.

The two degradation products detected are likely a quinone and a dimer. Based on this data, propofol contained in Formulations D and F is predicted to possess stability at room temperature for periods of time greater than 4 weeks. High temperature stability (i.e., at 40°C) of Formulation F indicates a projected propofol stability of about 1 to 2 years under refrigerated conditions.

Example 5

Propofol containing Formulation F was prepared as in Example 3. Samples of the compositions were separately sealed in glass vials and then were held at the temperatures indicated in Table 5 for the indicated amount of time. HPLC analysis of the samples was performed using the methods of Example 4. Table 6 shows the total degradates as percent of peak area by HPLC measured in Formulation F as a function of time and temperature.

TABLE 6. Total degradates (percent of peak area by HPLC) in Formulation F as a function of time and temperature

Time	Temperature		
	25°C	40°C	60°C
4 weeks	None detected	None detected	None detected
8 weeks	<0.1	<0.1	<0.1
12 weeks	0.44	1.01	1.30

The data presented in Table 6 demonstrate that compositions of Formulation F are stable for at least three months.

Example 6

Propofol Formulations C and D were made having the same compositions, and prepared by the same methods, as Examples 1 (Formulation C) and 2 (Formulation D). These compositions, along with Diprivan® Injectable Emulsion (AstraZeneca) as a control, were then evaluated in vivo for pharmacokinetic profiles.

Adult male Sprague-Dawley rats were obtained from Charles River Canada, Inc. (St. Constant, Quebec, Canada). At the time of use, the animals each weighed about 250 to 290 grams. The overall design for the animal study is summarized in Table 7.

TABLE 7. *In vivo* Pharmacokinetic Study Design

Group	Formulation	Dose (mg/kg)	Dose Volume (mL/kg)	Number of Animals	Samples Collected
1	Control	10	1	4	Plasma
2				4	Blood
3	C	10	1	4	Plasma
4				4	Blood
5	D	10	1	4	Plasma
6				4	Blood

Formulations were administered to the animals by intravenous injection via a jugular vein. The formulations were administered at a dose volume of 1 mL/kg over a period of approximately 1 minute (slow push) via jugular venipuncture under isoflurane anesthesia. As shown in Table 7, each formulation was administered to 2 groups of 4 animals. Animals were randomly selected to fill the study groups on the basis of comparable body weights.

Following administration, blood samples (0.25 to 0.40 mL) were collected by jugular venipuncture under anesthesia from each of the animals at pre-dose (i.e., immediately following completion of dose administration), 2, 3, 5, 7, 10, and 15 minutes from the start of dose administration. The animals were maintained in dorsal recumbancy during both dose administration and during blood sampling.

Blood samples from groups 2, 4, and 6 were stored at -20°C nominal pending further analysis. Blood samples from groups 1, 3, and 5 were centrifuged at 3200 g at 4°C nominal for 10 minutes. The resulting plasma samples were harvested and stored at -20°C nominal pending further analysis.

The animals were observed constantly during dose administration and blood sampling. The time for the animals to regain ventral recumbancy was recorded as an indication of duration of anesthesia. Table 8 shows the mean time to first animal movement and the mean time to regain ventral recumbancy, along with standard deviations, for each of the formulations evaluated.

TABLE 8. Observations on the Effects of Anesthesia

Group	Formulation	Mean Time to First Movement (min) (S.D.)	Mean Time to Regain Ventral Recumbancy (min) (S.D.)
1 and 2	Control	11.6 (3.9)	17.5 (4.1)
3 and 4	C	13.5 (4.7)	15.6 (2.2)
5 and 6	D	10.8 (4.1)	14.4 (2.8)

All plasma and blood samples were analyzed for propofol concentration using LC-MS/MS. Pharmokinetic analysis of propofol in plasma and blood were performed using the PhAST software program (Version 2.3, Phoenix International Life Sciences, Inc, Saint-Laurent, Quebec, Canada).

The area under the concentration-time curve between 0 and 15 minutes (AUC_{0-15}) was lower in plasma following administration of the novel propofol compositions (i.e., Formulations C and D) relative to the emulsion control. Propofol clearance (CL) was relatively similar following administration of Formulations C and D and the control. A significant increase in the volume of distribution (Vss) was observed for Formulations C and D from the plasma data (Table 9) and reflects distribution of the drug from plasma into other tissues. An inverse correlation existed between the volume of distribution in plasma and the particle size of the formulations; the emulsion control had micrometer-size droplets while the novel propofol compositions' particles were below 100 nanometers in size. The blood data, obtained by assaying whole blood at each time point for the presence of drug, showed comparable parameters between formulations (Table 10) indicating mass balance of the drug at an equivalent dose. The combined data of

Tables 9 and 10 strongly suggest that the nature of the formulation, in particular particle size and availability of propofol to the aqueous medium, plays an important role in determining plasma-blood partitioning of this highly lipophilic drug.

TABLE 9. Mean, ±Standard Deviation, pharmacokinetic parameters of propofol in plasma following a single intravenous dose (10 mg/kg) of a novel propofol formulation (C or D) and a commercially available emulsion formulation in male Sprague-Dawley rats.

Parameter	Formulation C	Formulation D	Control Emulsion Formulation
AUC_{0-15} (mcg.min/mL)	14.4 ± 3.2 †	18.4 ± 2.2	31.1±8.9
CL (mL/min/kg)	456±113†	254±80	242±31
Vss (mL/kg)	5342±1145†	7338±2748	2595±612

† $p < 0.05$ vs Control Emulsion Formulation.

TABLE 10. Mean, ±Standard Deviation, pharmacokinetic parameters of propofol in blood following a single intravenous dose (10 mg/kg) of a novel propofol formulation and a commercially available emulsion formulation in male Sprague-Dawley rats.

Parameter	Formulation C	Formulation D	Control Emulsion Formulation
AUC_{0-15} (mcg.min/mL)	62.7 ± 16 †	60.2 ± 11	45.6±6.2
CL (mL/min/kg)	112±20†	88±27	192±30
Vss (mL/kg)	1516±596†	1820±550	1292±183

† $p < 0.05$ vs Control Emulsion Formulation.

Figure 6 shows the mean plasma and blood concentrations of propofol following administration of Formulations C and D and the Diprivan Emulsion control to the male rats.

Using historical values of red blood cell (RBC) counts in rats, calculations were performed to obtain the area under the concentration-time curve (AUC_{0-15}) and the plasma-RBC partition coefficient (K_p) for Formulations C and D, as examples of novel propofol compositions, and compared to calculations made for Diprivan® Injectable Emulsion. The fraction of propofol sequestered in RBC with Formulations C and D appear to be markedly higher than that of the emulsion formulation (Table 11). Figure 7 shows mean predicted propofol concentrations in red blood cells (RBC) versus time following single intravenous doses (10 mg/kg) of Formulation C and Diprivan® Injectable Emulsion in male rats. Following intravenous administration, it appears that propofol from the novel composition concentrates in lipid-rich areas of blood, which

participate in the uptake and transfer to its active site and provide anti-platelet and antioxidant activity during anesthesia. Since propofol affinity for whole blood and RBC is an important determinant on the onset, intensity and duration of anesthesia, the results support the hypothesis that the novel composition of propofol can enhance or even optimize the in vivo pharmacological activity of the drug. These results also indicate that additional benefits such as improved resistance of erythrocytes to physical and hemodynamic stress during anesthesia, a greater preservation of red blood cell counts after surgery, and a reduction of reperfusion injury in surgery may be associated with the use of the novel propofol compositions of the present invention.

TABLE 11. Calculated Mean ± Standard Deviation AUC_{0-15} and K_p of propofol in RBC following a single intravenous dose (10 mg/kg) of a novel propofol composition (Formulations C and D) and a commercially available emulsion formulation in male Sprague-Dawley rats.

Parameters	Formulation C	Formulation D	Diprivan® Injectable Emulsion
AUC_{0-15} (mcg.min/mL)	59.0±20.6†	59.7±22.3†	17.6±3.0
K_p (RBC:Plasma)	8.74±3.09†	6.31±0.89†	2.03±0.16

† $p < 0.05$ vs Diprivan® Injectable Emulsion.

Example 7

In vitro hemolysis of TPI-213F (1% w/w propofol, 5% w/w poloxamer 188, 4% w/w PEG 400, 1.5% w/w polysorbate 80, 1% w/w propylene glycol, and 2 mg/ml citric acid) was assessed using fresh human whole blood. This study was performed at MDS Pharma Services (Montreal, Canada). Blood was obtained from 2 human volunteers of mixed gender and compatible blood type. Blood samples were pooled and spiked with stock solutions of Diprivan® or TPI-213F in plasma to final concentrations of 10 ug/mL. A saline control was tested to establish auto-lysis of the red blood cells. All samples were incubated at 37°C. At 15, 45 min and 1, 1.5, and 2 hours post-onset of incubation, aliquots (in triplicate) of the whole blood were removed from each sample and centrifuged at 3,200 g for 10 min to obtain plasma. The plasma samples were analyzed for hemoglobin content by measuring the absorbance at 415 nm.

Visual appraisal of hemolysis prior to hemoglobin content determination indicated that there was evidence of hemolysis in all Diprivan® samples at 2 hour following onset of incubation. In contrast, no visual evidence of hemolysis was observed for any TPI-213F samples at any of the time points.

Mean concentrations of hemoglobin in plasma following incubation with increasing amount of Diprivan® and TPI-213F were measured. Consistent with visual appraisal observations, TPI-213F showed lower hemoglobin ($p<0.05$, the student's t test) concentrations at all time points compared to Diprivan®. This indicates that TPI-213F is milder on red blood cells than Diprivan®.

The hemoglobin concentration in plasma following incubation with the saline control establishes the baseline from auto-lysis of the red blood cell over time. Compared to this baseline, the TPI-213F related samples showed lower hemolysis ($p<0.025$, the

student's t test), indicating that the components in TPI-213F have a stabilizing effect on the red blood cell membrane. In contrast, all Diprivan® samples showed more hemolysis than saline at later time points (after 1 hr incubation, $p<0.05$).

Example 8

A pharmacokinetic study was carried out at MDS Pharma Services in beagle dogs (weight 8-10 kg) to compare TPI-213M (1% propofol w/v, 8% poloxamer 188 w/v, 3% PEG-400 w/v, 1% propylene glycol w/v, 20 mg/ml citric acid, 0.45% benzyl alcohol w/v) and RAPINOVET (a currently marketed lipid based emulsion). All animals were handled according to established guidelines and principles. Administration of all formulations was achieved via slow push over a period of about 1 min through an indwell catheter. All dogs received the same dosing regiment in a cross-over design as follows:

TABLE 12

Dosing Day	Formulation	Dose (mg/kg)	Dose Volume (mL/kg)	No. of Dogs	Sample collected
Day 1	TPI-213M	6	0.6	3	Plasma, blood
Day 1	Rapinovet	6	0.6	3	Plasma, Blood
Day 8	Rapinovet	6	0.6	3	Plasma, Blood
Day 8	TPI-213M	6	0.6	3	Plasma, blood

Following dose administration, blood samples were collected at various time points. An aliquot of blood was removed for analysis and the remaining blood was centrifuged at 3,200 g at 4°C for 10 min. The resulting plasma samples were harvested and stored at -20°C for analysis of propofol.

The pharmacokinetic parameters calculated for TPI-213M and Rapinovet are shown in Table 13. TPI-213M showed similar plasma concentrations compared to Rapinovet, suggesting that TPI-213M is bioequivalent to Rapinovet. Both formulations also showed similar propofol concentrations and AUC values in blood as in plasma, suggesting that there is no preferential partitioning of the drug into dog red blood cells from either formulation. This is different from what was seen in the rats, pointing to species-related differences in red blood cell partitioning.

Table 13. Mean pharmacokinetic parameters for propofol in plasma and blood following a single intravenous dose of Rapinovet or TPI-213M to Beagle Dogs. Values shown are mean±standard deviation.

Group	Test Article	Matrix	Dose (mg/kg)	$AUC_{(0-\infty)}$ (ng·hr/mL)	$t_{½}$ (hr)	V_{dss} (mL/kg)	CL (mL/hr·kg)
1	TPI-213M	Plasma	6	929±128	0.38±0.15	3056±539	6553±834
2	TPI-213M	Blood	6	746±249	0.36±0.07	4594±3511	9121±4200
3	Rapinovet	Plasma	6	1052±255	0.41±0.30	2509±1476	6051±1747
4	Rapinovet	Blood	6	892±320	0.29±0.03	2485±1297	7869±4111

$AUC_{0-\infty}$: The area under the concentration vs. time curve from time zero to infinity

$t_{½}$: Terminal phase half-life

V_{dss}: Apparent volume of distribution

CL: Plasma or blood clearance

During this pharmacokinetic study, the dogs were also observed for the pharmacological effect from the two formulations, i.e., time to sleep and time to full awakeness. The data suggests that TPI-213M has the same pharmacological effect as Rapinovet.